



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application Number : 09/767,680 Confirmation No.: 4425
Applicant : Asger GEPPEL *et al.*
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Title : PORPHYRIN CONTAINING LACTIC ACID BACTERIAL CELLS
AND USE THEREOF
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Commissioner for Patents
P.O. Box 1450
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DECLARATION UNDER 37 C.F.R. § 1.132

Sir,

I, Asger Geppel, declare that:

1) I have received a Bachelor of Science degree in chemical engineering from Danmarks Ingeniørakademi, Copenhagen, Denmark, in 1988.

2) I am a senior research scientist at, and am employed by, Chr. Hansen A/S, assignee of all right, title and interest in the present patent application. I have been associated with research in the field of analytical chemistry within the field of lactic acid bacteria for approximately 11 years.

3) I am a named inventor of U.S. Patent Application Serial Number 09/767,680. Based on the academic training and professional experience, I consider myself a person of

ordinary skill in the technology of lactic acid bacterial cells and modification thereof, and I was such a person in 2001 when the Application was filed.

- 4) I have read, and am familiar with, the following documents:
 - a.) U.S. Patent Application No. 09/767,680 (hereinafter the "680 application");
 - and
 - b.) the Final Office Action mailed April 19, 2005, in the '680 application.

5) It is my understanding that claims 1, 4-17, 34-52, 56 and 57 of the '680 Application were rejected for the scope of enablement, because the specification allegedly does not reasonably provide enablement for the scope of the claimed subject matter. I have been asked to comment on whether the specification of the '680 Application is enabling for the claims that I understand will be submitted in response to the Final Office Action. A copy of such claims with the proposed amendments is attached as the Declaration Appendix.

6) As described at page 7, lines 3-19 of the '680 Application, "porphyrin compound" is a generic name for a group of cyclic tetrapyrrole derivative compounds whose structures are derived from that of porphyrin by substitution at the carbon atoms located at the apices of the pyrrole core with various functional groups, and in my opinion that is the understanding of a person of ordinary skill in the art. Porphyrin compounds include a tetrapyrrole ring, also referred to as a "tetrapyrrole ring system" and a "porphyrin ring". Porphyrin compounds are widely distributed in e.g. plants and animals. An example of a porphyrin compound is a tetrapyrrole ring system that combines with Fe^{2+} to form a haeme (or heme), or with Fe^{3+} (and the counter ion Cl^-) to form haemin, which are iron containing porphyrins. Thus, haemin is the chloride salt of a specific haeme compound (haemin = haeme⁺ - Cl^-). We found that when lactic acid bacterial (hereinafter "LAB") cells are treated with a fermentation medium (also referred to herein as a "fermentation medium substrate" or a "medium"), containing a porphyrin compound which includes iron, such as haemin, the cells comprise a porphyrin compound which includes iron.

7) "Iron containing porphyrin compounds" (also referred to in this Declaration as "porphyrin compounds which include iron") are iron complexed forms of porphyrin compounds.

Haeme is an example of an "iron containing porphyrin compound". A haeme molecule is bound to the protein backbone of cytochromes. In blood a haeme molecule is bound to the protein backbone of hemoglobin. The term "iron containing porphyrin compound" comprises, but is not limited to, haeme, haemin, cytochrome, and hemoglobin.

8) In my opinion, when the iron containing porphyrin compound added to the medium has a "free" iron containing porphyrin ring not connected with a protein, such as haeme or haemin, the resulting LAB cells comprise the free iron containing porphyrin ring, both when grown anaerobically and aerobically (*See Specification, Table 4*). In my opinion, it is clear to the skilled person that in order to make LAB cells which comprise an iron containing porphyrin compound, one does not have to use the "pure" haemin (haeme salt, *see above*) product, but could as well use haeme proteins from animal sources such as blood.

9) It is also my opinion that when an iron containing porphyrin compound, in such a form that it is connected with a protein, e.g., hemoglobin, is added to the fermentation medium substrate, the iron containing porphyrin ring separates from the protein backbone and can easily be included in the LAB cells. In my opinion, it is also known to the skilled person that haeme (an iron containing porphyrin ring) is relatively loosely bound in most cytochromes or hemoglobin. Accordingly, when blood is added to a fermentation media the hemoglobin separates into the haeme and the rest of the globin structure (or haeme can be easily liberated as it is not covalently bound to the protein backbone).

10) As taught by the specification, when LAB cells are grown in the presence of an iron containing porphyrin compound under aerobic conditions, the LAB cells form a cytochrome (*See Specification as filed, e.g., page 7, line 33 extending to page 8, line 2; and Experiment 2, results, page 28*). In my opinion, the LAB cells form a cytochrome by combining the added iron containing porphyrin ring with a protein, thereby restoring the respiratory chain. Further as taught in the specification in Experiment 2 and page 28, lines 24-32, it is demonstrated that the cells fermented under aerobic conditions comprise cytochrome *d*, although cytochrome *d* is not added to the fermentation media. I conclude that the LAB cells (when grown aerobically) not

only comprise the added porphyrin ring but also make a cytochrome that comprises the porphyrin ring.

11) In the examples of the specification, the LAB cells comprise an iron containing porphyrin compound when an iron containing porphyrin compound, such as haemin, is added to the fermentation medium under aerobic and anaerobic conditions. Also, the LAB cells comprised cytochrome d when the cells were fermented under aerobic conditions (*See* Specification as filed, Example 1, Experiment 2). In my opinion, by demonstrating that iron containing porphyrin compounds are comprised by the cells when they are grown in a medium containing haemin, the inventors have enabled the invention for all iron containing porphyrin compounds. In particular, based on the specification, including the examples, it is my opinion that I would be able to make LAB cells comprising an iron containing porphyrin ring when cultured in a fermentation medium containing any of the iron containing porphyrin compounds in any effective amounts desirable, without undue experimentation, if any were needed. I use the term "effective amount" to have the same meaning as in the '680 application, i.e., "an amount that is sufficient to cause the lactic acid bacterium to become modified", e.g., *See* page 6, lines 7-8.

12) Since all types of iron containing porphyrin compounds are closely related (*See* specification, page 7, lines 14-16), it is my opinion that the invention should work for all types of iron containing porphyrin compounds. Further, the specific source of the iron containing porphyrin compound added to the fermentation medium is not relevant.

13) The examples in the specification utilized haemin as the source of the iron containing porphyrin compound. However, blood containing extract could have been used as well because, in my opinion, all that is necessary is a suitable iron containing porphyrin compound present in an effective amount in the LAB fermentation medium in order to allow the LAB cells to comprise the iron containing porphyrin ring and maintain it. A starter culture of individual LAB cells comprising an iron containing porphyrin ring may then be harvested from the media.

14) As discussed previously, all iron containing porphyrin compounds (iron complexed forms) are closely related. In my opinion, a person of ordinary skill knows that different iron containing porphyrin compounds share the same chemical core structure. Therefore, if the LAB cells (treated as discussed above and in the specification with an iron containing porphyrin compound derived from animal sources, e.g., haemin) can comprise and maintain an iron containing porphyrin ring derived from that or a similar iron containing porphyrin compound, in my opinion the person skilled in the art would understand that LAB cells can comprise and maintain an iron containing porphyrin ring derived from other sources (e.g., synthetically made), and work in a similar way.

15) The undersigned acknowledges that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. 1001), and may jeopardize the validity of the application or any patent issuing thereon. The undersigned declares further that all statements made herein of his/her own knowledge are true and that all statements made on information and belief are believed to be true.

I declare under penalty of perjury that the foregoing is true and correct.

Executed on 23. sep. 2005

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Declaration Appendix

Claims:

1-3. (Canceled)

4. **(Currently Amended)** A The composition according to claim 13, wherein the modified lactic acid bacterial cell according to claim 1 that contains comprises at least 0.1 ppm on a dry matter basis of a cytochrome.

5. **(Currently Amended)** A The composition according to claim 13, wherein the modified lactic acid bacterial cell according to claim 1 that contains comprises at least 0.1 ppm on a dry matter basis of cytochrome *d*.

6. **(Currently Amended)** A The composition according to claim 13, wherein the modified lactic acid bacterial cell according to claim 1 which is of a bacterial species selected from the group consisting of *Lactococcus* spp., *Lactobacillus* spp., *Leuconostoc* spp., *Pediococcus* spp., *Streptococcus* spp., *Propionibacterium* spp., *Bifidobacterium* spp., and *Oenococcus* spp.

7. **(Currently Amended)** A The composition according to claim 13, wherein the modified lactic acid bacterial cell according to claim 6 where the bacterial species is of *Lactococcus lactis*.

8. **(Currently Amended)** A The composition according to claim 13, wherein the modified lactic acid bacterial cell according to claim 1 which, when it is in the form of a cell suspension, is inoculated in a concentration of 10^7 cells/ml into low pasteurised skimmed milk having 8 ppm of dissolved oxygen and the milk is allowed to stand for about two hours at a temperature of about 30°C, the cell consumes at least 25% of the dissolved oxygen.

9. **(Currently Amended)** A The composition ~~modified lactic acid bacterial cell~~ according to claim 8 where the modified lactic acid bacterial cell consumes at least 50% of the dissolved oxygen.

10. **(Currently Amended)** A The composition according to claim 13, wherein the modified lactic acid bacterial cell ~~according to claim 1, which,~~ relative to a cell from which it is derived, has a decreased NADH oxidase (NOX) activity, a decreased lactate dehydrogenase (LDH) activity, or a decreased NOX activity and decreased LDH activity.

11. **(Currently Amended)** A The composition according to claim 10, wherein the modified lactic acid bacterial cell ~~according to claim 10 that~~ has a NOX activity which is decreased by at least 10% under aerobic conditions.

12. **(Currently Amended)** A The composition according to claim 10, wherein the modified lactic acid bacterial cell ~~according to claim 10 that~~ has a LDH activity which is decreased by at least 10%.

13. **(Currently Amended)** A starter culture composition useful in manufacturing and preservation of food and feed products, comprising the a modified lactic acid bacterial cell of claim 1 that has been treated with a medium comprising a porphyrin compound which includes iron to cause said modified lactic acid bacterial cell to comprise at least 0.1 ppm on a dry matter basis of a porphyrin compound which includes iron, wherein said modified lactic acid bacterial cell is harvested following treatment with said medium comprising a porphyrin compound which includes iron and is useful as a starter culture for the manufacture of food and feed products.

14. **(Currently Amended)** A The composition according to claim 13, wherein the composition is in the form of a frozen, liquid or freeze-dried composition.

15. **(Currently Amended)** A The composition according to claim 13 comprising an amount of viable modified lactic acid bacterial cells which is in the range of 10^4 to 10^{12} CFU per gram.

16. **(Currently Amended)** A The composition according to claim 13 which comprises modified lactic acid bacterial cells of two or more different lactic acid bacterial strains.

17. **(Currently Amended)** A The composition according to claim 13, ~~which further comprises~~ further comprising at least one component ~~enhancing~~ which enhances the viability of the modified lactic acid bacterial cell during storage.

18-28. (Canceled)

29. (Withdrawn) A method of reducing the oxygen content in a food or feed product or in a food or feed product starting material comprising adding to the product or to the starting material an effective amount of the starter culture composition according to claim 13.

30. (Withdrawn) A method of improving the shelf life and/or the quality of an edible product comprising adding to the product an effective amount of the starter culture composition according to claim 13.

31. (Withdrawn) A method of preparing a fermented food or feed product, comprising adding an effective amount of the composition of claim 13 to a food or feed product starting material, wherein the composition is capable of fermenting said starting material to obtain the fermented food or feed product.

32. (Withdrawn) Use of the composition of claim 13 for the production of a metabolite produced by the composition or by a non-modified cell co-cultivated therewith.

33. (Withdrawn) Use of the composition of claim 13 for the production of a bacteriocin.

34. (Currently Amended) ~~A modified lactic acid bacterial cell according to claim 6,~~
~~where~~ The composition of claim 13, wherein the bacterial species of the lactic acid bacterial cell
to be modified is *Lactococcus lactis* strain CHCC373 deposited under the accession number
DSM12015.

35. (Currently Amended) A The composition according to claim 13, which includes a
bacterial nutrient, a cryoprotectant or a bacterial nutrient and a cryoprotectant.

36. (Currently Amended) A The composition according to claim 13, wherein the
modified lactic acid bacterial cell ~~according to claim 1 which contains~~ comprises at least 0.2 ppm
on a dry matter basis of ~~[[a]]~~ the porphyrin compound which includes iron.

37. (Currently Amended) A The composition according to claim 13, wherein the
modified lactic acid bacterial cell ~~according to claim 1 which contains~~ comprises at least 1 ppm
on a dry matter basis of ~~[[a]]~~ the porphyrin compound which includes iron.

38. (Currently Amended) A The composition according to claim 13, wherein the
modified lactic acid bacterial cell ~~according to claim 1 which contains~~ comprises at least 5 ppm
on a dry matter basis of ~~[[a]]~~ the porphyrin compound which includes iron.

39. (Currently Amended) A The composition according to claim 13, wherein the
modified lactic acid bacterial cell ~~according to claim 1 which contains~~ comprises at least 20 ppm
on a dry matter basis of ~~[[a]]~~ the porphyrin compound which includes iron.

40. (Currently Amended) A The composition according to claim 13, wherein the
modified lactic acid bacterial cell ~~according to claim 1 which contains~~ comprises at least 60 ppm
on a dry matter basis of ~~[[a]]~~ the porphyrin compound which includes iron.

41. **(Currently Amended)** A The composition according to claim 13, wherein the modified lactic acid bacterial cell ~~according to claim 1 which contains~~ comprises at least 80 ppm on a dry matter basis of ~~[[a]]~~ the porphyrin compound which includes iron.

42. **(Currently Amended)** A The composition according to claim 13, wherein the modified lactic acid bacterial cell ~~according to claim 1 which contains~~ comprises at least 100 ppm on a dry matter basis of ~~[[a]]~~ the porphyrin compound which includes iron.

43. **(Currently Amended)** A The composition according to claim 13, wherein the modified lactic acid bacterial cell ~~according to claim 1 which contains~~ comprises at least 0.5 ppm on a dry matter basis of a cytochrome.

44. **(Currently Amended)** A The composition according to claim 13, wherein the modified lactic acid bacterial cell ~~according to claim 1 which contains~~ comprises at least 10 ppm on a dry matter basis of a cytochrome.

45. **(Currently Amended)** A The composition according to claim 13, wherein the modified lactic acid bacterial cell ~~according to claim 1 which contains~~ comprises at least 40 ppm on a dry matter basis of a cytochrome.

46. **(Currently Amended)** A The composition according to claim 13, wherein the modified lactic acid bacterial cell ~~according to claim 1 which contains~~ comprises at least 70 ppm on a dry matter basis of a cytochrome.

47. **(Currently Amended)** A The composition according to claim 13, wherein the modified lactic acid bacterial cell ~~according to claim 1 which contains~~ comprises at least 90 ppm on a dry matter basis of a cytochrome.

48. **(Currently Amended)** A The composition according to claim 13, wherein the modified lactic acid bacterial cell according to claim 1 which reduces the amount of oxygen present in a medium by at least 1% per hour.

49. **(Currently Amended)** A The composition according to claim 13, wherein the modified lactic acid bacterial cell according to claim 1 which reduces the amount of oxygen present in a medium by at least 20% per hour.

50. **(Currently Amended)** A The composition according to claim 13, wherein the modified lactic acid bacterial cell according to claim 1 which reduces the amount of oxygen present in a medium by at least 40% per hour.

51. **(Currently Amended)** A The composition according to claim 13, wherein the modified lactic acid bacterial cell according to claim 1 which reduces the amount of oxygen present in a medium by at least 70% per hour.

52. **(Currently Amended)** A The composition according to claim 13, wherein the modified lactic acid bacterial cell according to claim 1 which reduces the amount of oxygen present in a medium by at least 90% per hour.

53. **(Withdrawn)** A method for the production of a metabolite comprising adding the composition of claim 13 to a starting material and maintaining the resulting mixture under conditions suitable to produce the metabolite.

54. **(Withdrawn)** A method for the production of a metabolite comprising adding the composition of claim 13 and a non-modified cell co-cultivated with the composition and maintaining the resulting mixture under conditions suitable to produce the metabolite.

55. (Withdrawn) A method for the production of a bacteriocin comprising adding the composition of claim 13 to a starting material and maintaining the resulting mixture under conditions suitable to produce bacteriocin.

56. (Currently Amended) A The composition according to claim 13, wherein the modified lactic acid bacterial cell of claim 1 which exhibits a modified aerobic breakdown of carbohydrates as compared to a lactic acid bacterial cell which has not been treated with the medium comprising a porphyrin compound which includes iron.

57. (Canceled)